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RELATIONSHIPS BETWEEN HEXACHLOROETHANE OBSCURANT, ZINC DEPOSITION, AND FOLIAR INJURY OF SEVERAL FOREST TREE SPECIES

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Open-top chambers were used to determine the relationships between a hexachloroethane (HC) obscurant, zinc deposition, and foliar injury of tree species indigenous to military training facilities. Eight eastern U.S. tree species were exposed four times to three exposure regimens (0x, 1x, 2x) of the obscurant, measured as Zn, during the 1990 growing season. Plots received ambient or charcoal-filtered air for comparison purposes. Black locust and black cherry were the most sensitive species, exhibiting significant ($p < 0.05$) necrotic leaf spot, chlorotic mottle, and marginal necrosis of leaves at the 2x levels throughout the season. Defoliation was also greater ($p < 0.05$) in exposed versus unexposed plots. Sugar maple injury was different among exposure regimens ($p < 0.05$) only near the end of the season. Sweet gum, eastern white pine, loblolly pine, and Virginia pine were asymptomatic. Charcoal filtration did not affect severity of symptoms. Zinc deposition was positively correlated ($p < 0.05$) with most of the symptoms on black locust and black cherry. The HC obscurant in the concentrations used in this study adversely affected these species.

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PREFACE

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RELATIONSHIPS BETWEEN HEXACHLOROETHANE OBSCURANT, ZINC DEPOSITION, AND FOLIAR INJURY OF SEVERAL FOREST TREE SPECIES

1. INTRODUCTION

Chemical smokes/obscurants are used by the Army to screen troop movements during training exercises at military testing facilities throughout the United States. One such material, hexachloroethane (HC) obscurant, is produced by reaction as follows:

$$C_2Cl_6 + 3ZnO + 2Al \rightarrow 3ZnCl_2 + Al_2O_3 + 2C + \text{heat}$$

Zinc chloride, the predominant product of this reaction, forms the desired smoke particles and through deposition may adversely affect vegetation directly or concentrate in vegetative tissue over time. Visible injury to foliage could be used as a bioindicator of phytotoxicity.

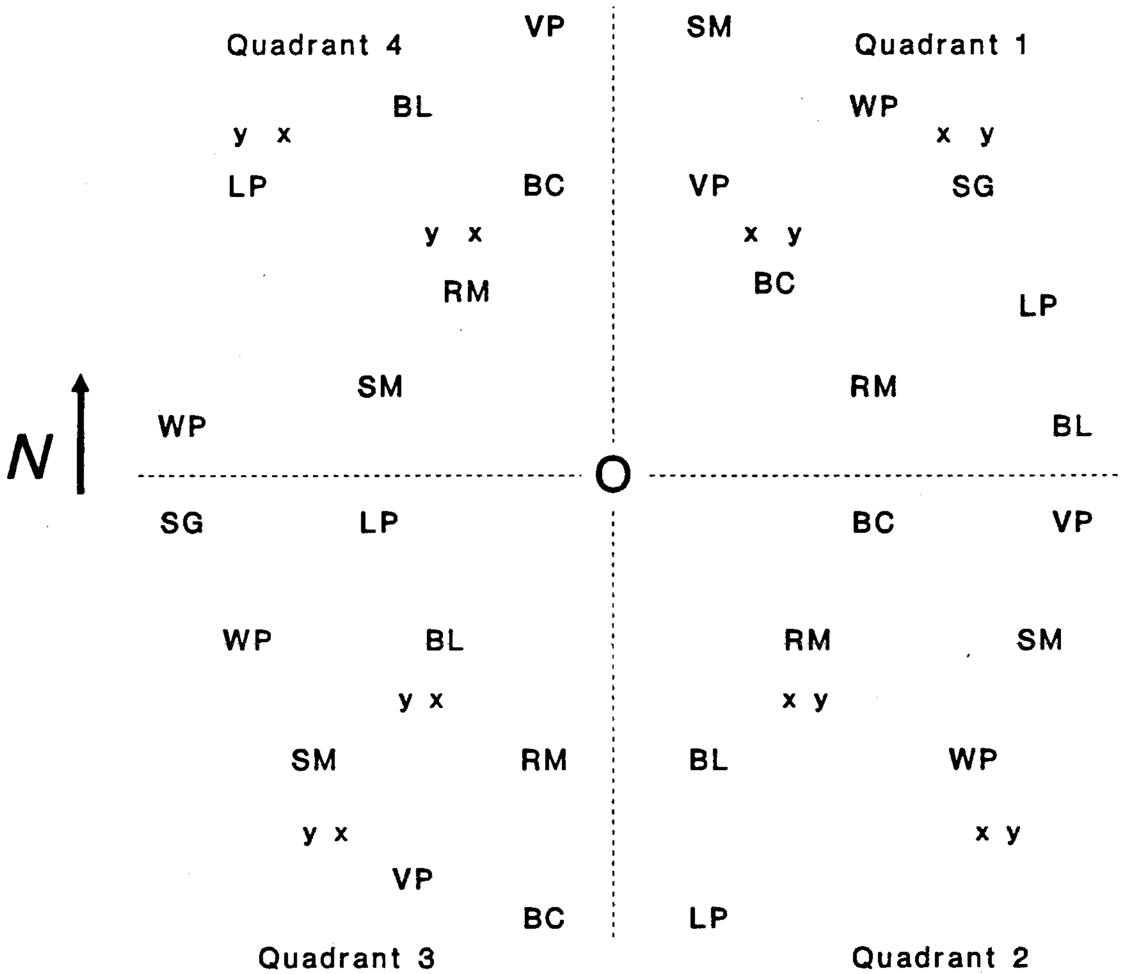
Previous studies have described foliar symptoms on several plant species exposed to smokes/obscurants in wind tunnel facilities [1-4]. In general, injury increased as exposure concentrations increased. However, these studies were conducted under laboratory conditions with containerized plants. Similar studies under field conditions have not been performed. Consequently, open-top chamber technology was investigated and found to be an effective tool for assessing obscurant phytotoxicity in the field [5,6].

The purposes of this study were to utilize open-top chambers to evaluate the phytotoxicity of HC obscurant on forest tree species indigenous to several eastern U.S. Army testing facilities, and to determine the relationship of foliar injury of these species to Zn deposition throughout the growing season.

2. MATERIALS AND METHODS

Tree seedlings were exposed at a testing site of the Edgewood area of Aberdeen Proving Ground, (MD) in the summer of 1990. This grassland site was predominantly a Sassafras sandy loam (fine-loamy, siliceous, mesic Typic Hapludult) that had high available water capacity. The site was fenced for deer exclusion and planted with tree species indigenous to eastern U.S. forests, including five broadleaf species - black locust (*Robinia pseudoacacia* L.), black cherry (*Prunus serotina* Ehrh.), sugar maple (*Acer saccharum* Marsh.), red maple (*Acer rubrum* L.), and sweet gum (*Liquidambar styraciflua* L.) and three coniferous species - Eastern white pine (*Pinus strobus* L.), loblolly pine (*Pinus taeda* L.), and Virginia pine (*Pinus virginiana* Mill.).

Sixteen individual circular plots, divided into four quadrants, were established. Each quadrant was planted with one two-year-old seedling of each species, except for sweet gum, which was planted in two quadrants only. Each plot diameter was 3.0 m, consisting of an inner-circle diameter of 1.2 m with trees planted 0.38 m apart and an outer-circle diameter of 2.1 m with trees planted 0.33 m apart. The trees were planted into the field soil and mulched with bark chips to reduce weed competition. The planting design for each of the 16 plots is presented in Figure 1.



BC ▪ black cherry (*Prunus serotina* Ehrh.)
BL ▪ black locust (*Robinia pseudoacacia* L.)
LP ▪ loblolly pine (*Pinus taeda* L.)
RM ▪ red maple (*Acer rubrum* L.)
SG ▪ sweetgum (*Liquidamber styraciflua* L.)
SM ▪ sugar maple (*Acer saccharum* Marsh.)
VP ▪ Virginia pine (*Pinus virginiana* Mill.)
WP ▪ white pine (*Pinus strobus* L.)
x ▪ location of dishes 0.15 m above the soil
y ▪ location of dishes 1.2 m above the soil
O ▪ HC obscurant canister placement

Fig. 1. Planting Design for Open-Top Chamber Plots with Locations of Plastic Dishes Used to Collect Obscurant Particles.

2.1 Chamber Structure/Experimental Design

Open-top chambers [7] were built to enclose each of 12 plots, consisting of an aluminum channel frame 2.4 m tall with a cylindrical diameter of 3.0 m. Each frame was covered with two clear PVC panels. The bottom panel contained a movable flap for entry/exit and was double-walled, with numerous perforations on the inner wall to allow air movement across the trees contained therein. A 0.5-m-diameter molded tube connected the bottom panel to a galvanized steel fan box that could be supplied with charcoal filters to "clean" the incoming air stream. A single-walled top panel acted as a short chimney, preventing incursion of ambient air over plants. The chambers had no covering across their tops, thus allowing air exchange to take place without buildup of heat or major changes in relative humidity, light, or rainfall availability to seedlings. The chambers were tied down with eyebolts, steel cable, and screwdown stakes to prevent movement during high winds.

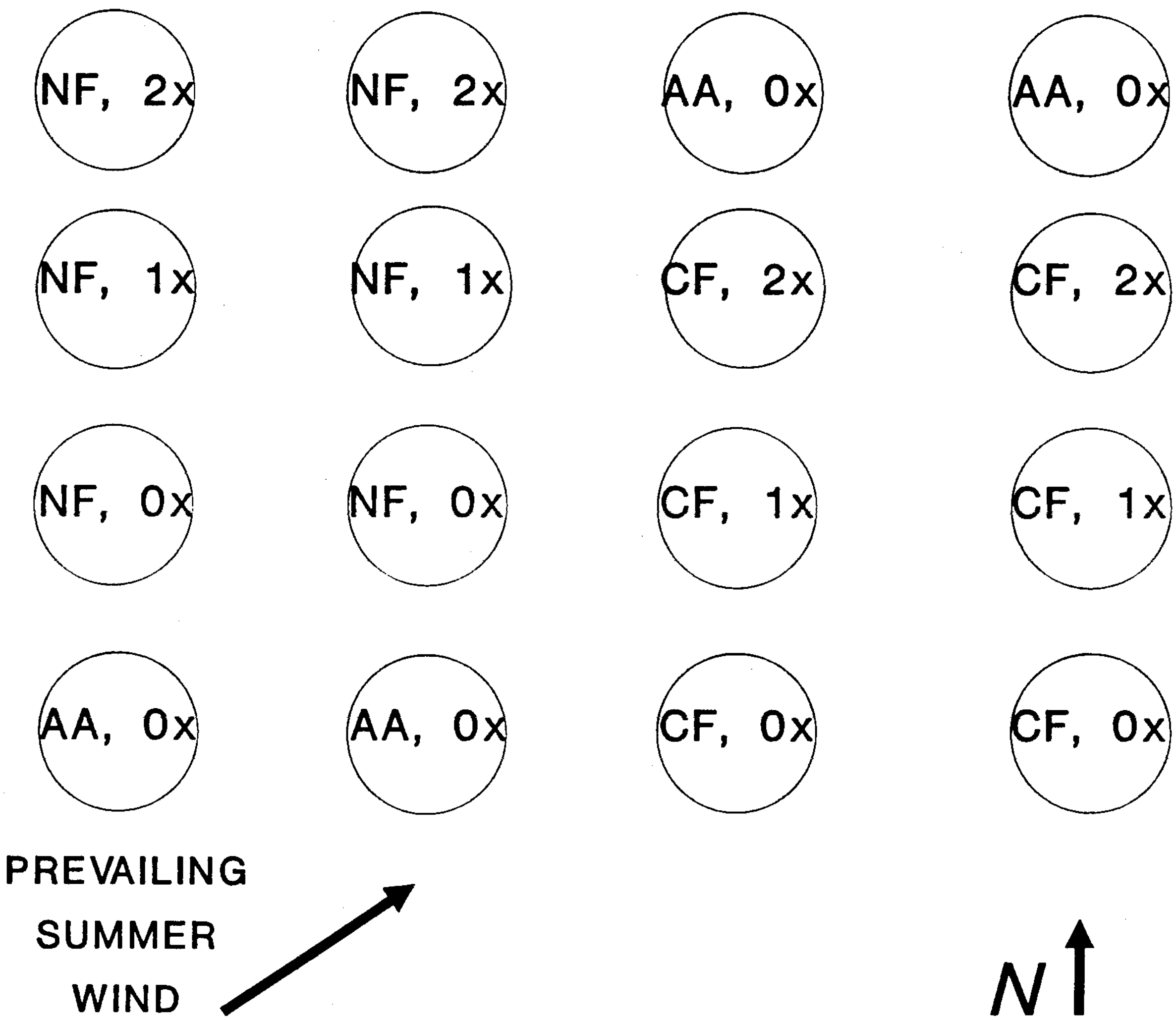
Ambient air was pulled through a roughing filter and, where designated (Fig. 2), twelve 61- x 61- x 1.9-cm activated charcoal filters by a 4,065-cfm fan and 0.75-hp motor in the fan box. It then passed through the molded tube into the bottom panel, from which it entered the chamber. A positive pressure caused the air to exit the chamber via the open top. Chamber operation commenced in the spring of 1990.

The experimental design included three exposure regimens (0x, 1x, 2x) and three air treatments (charcoal-filtered [CF], nonfiltered [NF], and ambient [AA] [i.e., no chamber]) (Fig. 2). Plots were located so that exposure intensity increased from upwind to downwind locations (Fig. 2) because 0x and 1x plots located downwind of other exposed plots might receive greater concentrations of obscurant than those located upwind, causing biased results. Soil type generally did not vary across the site, and any impacts due to minor differences in soil characteristics were estimated to be orders of magnitude less than those due to potential particle drift from exposures. Two AA plots were placed downwind as indicators of the phytotoxicity, if any, from this drift. Two replications of the CF and NF air treatments for each exposure regimen utilized the 12 chambers. The CF and NF treatments were used to distinguish foliar injury caused by the obscurant from injury induced by gaseous pollutants (i.e., O_3, SO_2) possibly present in ambient air.

2.2 HC Obscurant Composition/Exposure Monitoring

The exposure regimens utilized HC obscurant canisters, where "0x", "1x", and "2x" represented zero, one, and two canisters, respectively. Each canister contained a 539-g mixture consisting of 46.66% hexachloroethane, 46.66% zinc oxide, and 6.68% grained aluminum by weight [8]. Upon burning, 76.3% zinc chloride, 19.1% aluminum oxide, 4.5% carbon, and <1% other inorganic and organic constituents (arsenic, cadmium, iron, lead, aromatic hydrocarbons, and chlorinated aliphatics) were generated [9]. Zinc chloride was the major product; therefore, elemental Zn deposition was used to quantify the level of obscurant deposited on tree seedlings.

Trained personnel tossed ignited canisters into a 20-L metal container



AA = ambient air, no chamber
NF = non-filtered air, chambered
CF = filtered air, chambered

0x = no obscurant
1x = 1 canister
2x = 2 canisters

Fig. 2. Experimental Design for Open-Top Chamber Site.

sunk into a 1-m deep hole in the center of each exposed plot. Soil was placed around the rim of the hole, and a pointed metal cap was placed on the soil to protect the seedlings and chamber walls from heat and ignition sparks. So that 2x (two canister) exposure times would approximate those of 1x (single canisters), the two canisters were tossed into the metal container simultaneously.

Particle deposition was monitored via 8.5-cm-diameter plastic dishes placed at 16 locations within each chamber. Dishes were laid on both 0.15- and 1.2-m-tall wooden platforms, that is, near the ground and within the foliage, respectively, and were placed in the inner or outer circle of trees (Fig. 1). The dishes were keyed to their location within a plot for correlation of deposition and foliar injury data.

To minimize particle drift, fans were kept running in all chambers, except for the chamber receiving the obscurant. The fan was shut off immediately before canister ignition to allow maximum residence time for obscurant particles. After a completed canister burn (averaged 3.3 $\pm$ 1.5 min), the plastic dishes were covered and removed, and the fan was restarted. The dishes were returned to the laboratory, washed with dilute HNO_3 into volumetric flasks, diluted to 25 ml with distilled/deionized H_2O, and analyzed for Zn using atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, CT). Quality control for Zn analyses was obtained by using blanks, duplicates, and spikes to determine background Zn levels, instrumental accuracy, and percentage of recovery.

2.3 Environmental Conditions

Weather conditions were recorded on days when trees were exposed to HC obscurant. All exposures were conducted on partly cloudy afternoons between 1300 and 1400 h, where temperatures ranged from 22 to 29°C (averaged 26°C) and relative humidity ranged from 56 to 83% (averaged 70%). Wind speed varied from 6 to 37 km/h. During exposures, winds were from the north/northwest on the first two dates and from the south/ southwest on the second two dates. Prevailing winds are out of the west to northwest in the spring and become more southerly during the summer [10]. Wind direction was noted to account for variability in deposition in the plastic collection dishes due to the direction of ignition plumes and any resultant particle drift.

2.4 Seedling Response Measurements

Trees were exposed on June 11 and 25, and July 12 and 30, 1990. Symptoms were recorded for all plots on these dates, prior to and following exposure, and then on June 12, 18, and 26; July 3, 13, 24, and 31; and August 16. Seedlings were scored in two ways: average percentage of leaves per tree (% lvs/tree) exhibiting the symptom, and average percentage of area per leaf (% area/leaf) exhibiting the symptom. In this manner, any individual seedling was evaluated and scored for several symptom types in response to the exposures. Scoring increments were in five percentiles from 0 to 100%. Scores were averaged by plot, species, and symptom type. Average scores per treatment (two replications) were computed for each

species and symptom type. Notes were also taken on other types of symptoms unrelated to smoke exposure, such as insect attack, fungal infections, and O_3 exposure [11], to provide additional explanation of observed trends.

Samples of immature (new growth) and fully matured (old growth) leaf tissue were collected before exposure and on June 18, July 3 and 24, and August 7 for Zn and Cl analyses (in progress). New growth (growth following exposure dates), especially of rapidly growing species such as black locust and sweet gum, confounded evaluations between exposures. Estimates of percentage of leaves showing symptoms varied accordingly. No change was also recorded during evaluations to ensure each seedling was evaluated for even subtle changes in symptom expression. Specific symptoms for this study were defined as

1. Necrotic leaf spot (NLS) - bifacial necrotic lesions scattered across leaf tissue, i.e., not marginal.
2. Chlorotic mottle (CM) - interconnected pattern of yellow and green leaf tissue within leaf margins.
3. Marginal leaf necrosis (MLN) - bifacial necrosis of tissue at the margins of leaves.
4. Defoliation - early season leaf senescence, considered to be premature and induced by stress.

2.5 Statistical Analyses

Mean Zn deposition within each plot was averaged by exposure regimen (0x, 1x, 2x) for each exposure date, as was cumulative deposition from the four dates (Table 1). Raw data were log-transformed before statistical analyses to ensure a normal population distribution. A general linear models (GLM) factorial analysis (exposure regimen, 2 d.f.; air treatment, 2 d.f.; exposure regimen x air treatment, 2 d.f.; error, 57 d.f.) [12] was performed to delineate variation among exposure regimen and air treatment means for each symptom within each date. No significant differences ($p < 0.05$) were found among means for CF, NF, and AA; therefore, these means were pooled for further analyses. Bonferroni t tests were then performed to find significant differences among exposure regimen means.

Due to inherent variability of $ZnCl_2$ production among individual canisters upon burning, Zn deposition between chambers was somewhat varied. To account for this variability and to quantify more accurately the relationship between Zn deposition and foliar injury, Pearson's correlation coefficients [12] were calculated between each injury variable and Zn deposition. Zinc levels recorded within a given chamber (milligrams per plot) on June 11, June 25, July 12, and July 30 were matched with visual symptoms (% lvs/tree and % area/leaf) on June 18, July 3, July 24, and August 16, respectively.

3. RESULTS

Zinc deposition is shown in Table 1. Mean cumulative Zn deposition on July 30 (the sum of the mean deposition of the four HC smoke exposures) was

Table 1. Mean Zn Deposition in Plots Exposed to 0x, 1x, and 2x HC Obscurant, Expressed as Cumulative for the Season and by Exposure Date (1990).

	Zn deposition (mg/plot) on rating date			
	6/11	6/25	7/12	7/30
As cumulative for the season				
0x	22	27.5	33.4	41.9
1x	452	759	1,178	1,457
2x	513	1,231	1,999	2,783
By Exposure Date				
0x	22	5.5	5.9	8.5
1x	452	307	419	279
2x	513	718	768	784

approximately twice as great in the plots exposed to two canisters (2x) than that of plots exposed to one canister (1x). On three of the four individual exposure dates, deposition in the 2x plots was considerably greater than that in the 1x plots. More variability occurred in the 1x plots. Exposures were not distinctly differentiated into 1x and 2x on June 11 due to sporadic burning of canisters in two of the 2x plots.

3.1 Foliar Injury

The predominant symptoms on broadleaf species included NLS, CM, and MLN. Leaves with greatest injury eventually abscised before the end of the study. Symptoms occurred mostly on the older (fully matured) leaves throughout the exposures. Mild CM developed on leaves that emerged following smoke exposures on several seedlings of black cherry, black locust, and sugar maple, which suggests translocation of a phytotoxic chemical(s) from older to younger leaf tissues or subsequent root absorption. Foliar analyses will determine the magnitude of translocation of Zn and Cl ions. Conifers remained asymptomatic throughout the entire exposure sequence.

In a few cases in which the metal cap did not offer total protection from canister ignition heat, seedlings nearest the ignition site exhibited symptoms of heat-induced injury (scorched leaf tips and margins). Some foliage of conifers and black locust was coated with an unidentified dark oily substance, presumably from incomplete combustion of smoke reactants [8]. The affected foliage died, but subsequent growth was asymptomatic. In other instances, black locust and, to a lesser extent, black cherry foliage exhibited blackening and severe leaf curl immediately following exposure. This symptom was clearly different from heat-induced symptoms and might have been caused by highly acidic compounds formed on the leaf surface [13]. Tissues so affected with this symptom necrosed within 24 h. Chemical analyses of residues on leaf surfaces are required to determine the exact cause(s) of this symptom.

Poor growth and severe defoliation on seedlings in the two NF 0x plots (see Fig. 2) were caused by presumable soil-related problems. These two plots were excluded from statistical analyses; soil-related injuries were not observed in any other plots. Means from obscurant-induced injuries (NLS, CM, MLN, and defoliation) were not significantly ($p < 0.05$) different between CF 0x and AA (0x) plots and were therefore combined as the 0x treatment for further statistical analyses. Ozone-induced injury (purplish stippling of the upper leaf surface [11]) was significantly ($p < 0.05$) less on black cherry seedlings receiving CF air than that on trees exposed to NF or AA among all exposures. Seasonal O_3 concentrations (Maryland Department of the Environment, personal communication) were sufficiently high to induce injury on black cherry grown in unfiltered (NF and AA) air (seasonal daily mean > 40 ppb) [14]. However, O_3-induced injury was easily distinguishable from obscurant-induced injuries, and consequently did not confound the results.

3.2 Specific Symptoms by Species

3.2.1 Black locust

This species was highly sensitive to HC obscurant. Symptoms were observed in the 1x and 2x plots 7 d after the first exposure. In the 2x treatment, seedlings had 5.6 NLS, 8.4 CM, and 16 MLN expressed as % lvs/tree (Fig. 3) on June 18. Injury was the same or slightly less on June 25, 14 d after the first exposure. This pattern was repeated after each exposure throughout the growing season in the 2x plots. Peak injury occurred on August 16, 17 d after the final exposure.

Trees exposed to the 1x treatment responded differently from those exposed to the 2x treatment. Chlorotic mottle decreased to nearly zero two weeks after the first exposure (Fig. 3). This decrease may be explained partly by an increase in defoliation or progression of the CM into NLS or MLN. The intensity of the NLS, CM, and MLN in the 1x plots was similar to that in the 2x treatment following the first exposure but was less than half as great following the second exposure (Fig. 3).

These patterns of foliar injury may be explained by examining the temporal trends of the Zn deposition. As previously described, the deposition in the 2x plots was relatively uniform after June 11 (Table 1). Although the trees appeared to recover two weeks after each exposure, the overall injury increased as the season progressed, probably due to consistently high deposition. Conversely, Zn deposition in the 1x plots was highly variable from date to date (Table 1). The intensity of visual symptoms on seedlings in the 1x plots tended to fluctuate in response to the Zn deposition (Fig. 3). The consistently high deposition in the 2x treatment appeared to affect the leaves cumulatively, whereas the lower and more variable deposition in the 1x treatment allowed the plants to recover.

3.2.2 Black cherry

This species appeared sensitive to HC obscurant with symptoms of CM and MLN evident 7 d following the first exposure. Black cherry foliage in the 2x plots had significantly ($p < 0.05$) more NLS than that in the 0x plots on July 3, 8 d after the second exposure, and on July 24, 12 d after

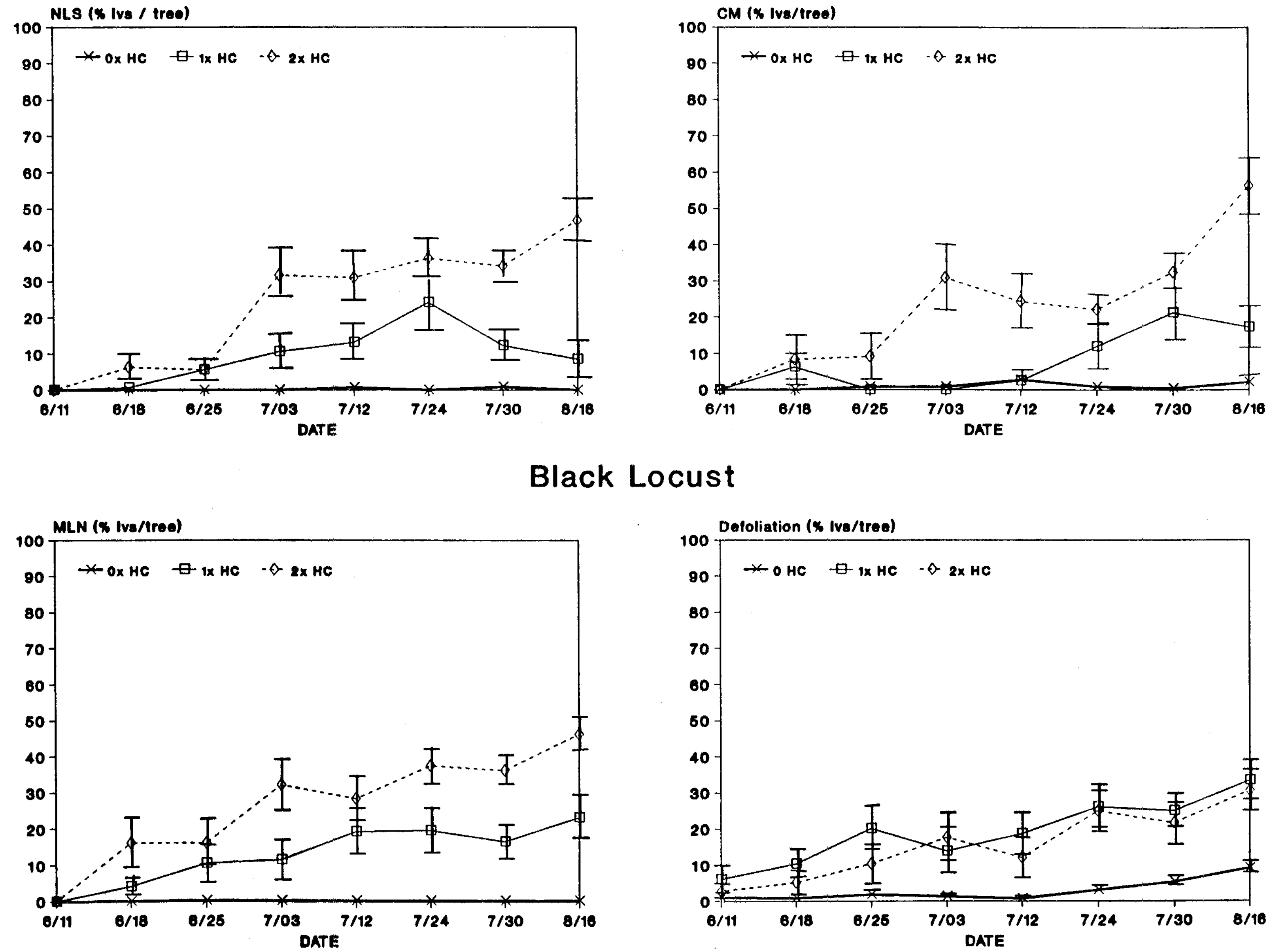


Fig. 3. Necrotic Leaf Spot (NLS), Chlorotic Mottle (CM), Marginal Leaf Necrosis (MLN), and Defoliation Expressed as Percentages of Leaves per Tree (% Lvs/Tree) on Black Locust Exposed to 0x, 1x, and 2x HC Smoke During the 1990 Growing Season.

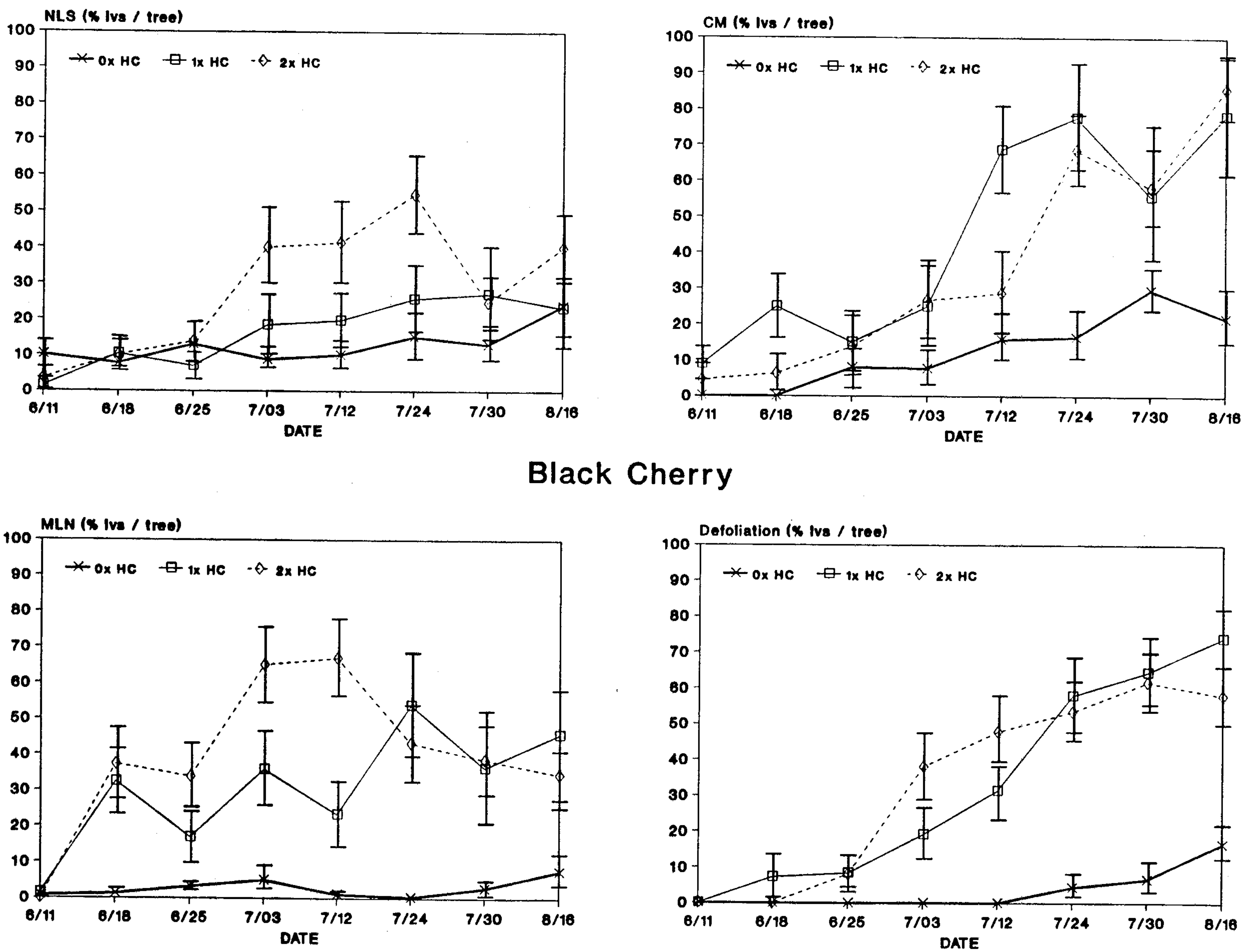


Fig. 4. Necrotic Leaf Spot (NLS), Chlorotic Mottle (CM), Marginal Leaf Necrosis (MLN), and Defoliation Expressed as Percentages of Leaves per Tree (% Lvs/Tree) on Black Cherry Exposed to 0x, 1x, and 2x HC Smoke During the 1990 Growing Season.

the third exposure (Fig. 4). Necrotic leaf spot (% lvs/tree) on trees in the 1x exposure did not differ significantly from that on the unexposed trees throughout the season. Chlorotic mottle remained similar among treatments until July 12 when it was significantly greater in the 1x plots than in the 0x plots (Fig. 4). Statistical significance was maintained in the exposed vs. unexposed plots through the end of the season, when CM reached its peak.

Of the observed symptoms on black cherry, MLN had the greatest difference between exposed and unexposed plots throughout the season (Fig. 4). Foliar injury in the 2x treatment peaked on July 12, then declined moderately. Defoliation of the exposed trees progressed steadily throughout the season and was significantly greater than defoliation of the untreated trees from July 3 until August 16 (Fig. 4).

Unlike black locust, for which NLS, CM, and MLN in the 2x plots increased as the season progressed, black cherry NLS and MLN decreased after July 24 and July 12, respectively. Leaves with these symptoms might have abscised prematurely on black cherry but remained attached on black locust.

3.2.3 Sugar Maple

Poor adaptability to high soil moisture content [15] contributed to high percentages of foliar injury that mimicked obscurant-related injury in the 0x and AA plots. However, greater foliar injury was observed in the exposed than in the unexposed plots throughout the growing season. These differences were statistically significant ($p < 0.01$) for NLS, CM, and MLN only on August 16 (data not shown); no detectable differences in defoliation by exposure regimen had occurred as of August 16.

3.2.4 Red Maple

Obscurant-induced injury was initially observed, but severe spider mite infestation prohibited accurate estimations of foliar injury due to exposures. The injuries were uniformly dispersed across exposed and unexposed plots, as well as AA plots. This species has therefore been excluded from further discussion.

3.2.5 Other Species

Sweet gum, eastern white pine, loblolly pine, and Virginia pine were all asymptomatic to HC obscurant exposures.

3.3 Correlation Analysis

Correlation coefficients of foliar injury vs. Zn deposition were determined for the most sensitive species - black locust and black cherry (Table 2). The NLS, CM, MLN, and defoliation on the black locust seedlings were positively correlated ($p < 0.05$) with Zn deposition from each of the exposures. Deposition was correlated more with injury expressed as % lvs/tree than with % area/leaf. Defoliation of black locust had the weakest relationship with Zn deposition (Table 2). Although all

Table 2. Pearson's Correlation Coefficients between Foliar Injury Variables and Zn Deposition by Date (1990).

	Correlation coefficient on rating date			
Injury variable	6/18	7/03	7/24	8/16
Black locust				
NLS (% lvs/tree)	0.46**	0.63**	0.57**	0.80**
NLS (% area/leaf)	0.41**	0.63**	0.48**	0.52**
CM (% lvs/tree)	0.37**	0.58**	0.43**	0.72**
CM (% area/leaf)	0.37**	0.40**	0.37**	0.35**
MLN (% lvs/tree)	0.57**	0.57**	0.65**	0.75**
MLN (% area/leaf)	0.29*	0.43**	0.35**	0.62**
Defoliation (% lvs/tree)	0.30*	0.28*	0.44**	0.37**
n =	56	56	56	56
Black cherry				
NLS (% lvs/tree)	0.27*	0.40**	0.35*	0.13
NLS (% area/leaf)	0.23	0.42**	0.28	0.39*
CM (% lvs/tree)	0.03	0.18	0.58**	0.51**
CM (% area/leaf)	0.11	0.11	0.34*	0.20
MLN (% lvs/tree)	0.39**	0.57**	0.43**	0.32*
MLN (% area/leaf)	0.30*	0.43**	0.47**	0.21
Defoliation (% lvs/tree)	0.21	0.49**	0.68**	0.35*
n =	56	55	47	41

Values under each rating date are correlation coefficients (r) between the Zn deposition on June 11, June 25, July 12, and July 30, 1990, and the foliar injury on the respective rating date. Deposition levels (milligrams per plot) in a given chamber were matched with % lvs/tree and % area/leaf within that chamber. NLS = necrotic leaf spot, CM = chlorotic mottle, MLN = marginal leaf necrosis. n = number of observations used to calculate the coefficient. *, ** indicate that the correlation between the injury variable and Zn deposition is not equal to zero at the 0.05 and 0.01 levels, respectively.

correlations between defoliation and deposition were significant($p < 0.05$), the maximum r value was only 0.44 on July 24.

In general, foliar injury on black cherry was not as highly correlated with deposition as it was with black locust (Table 2). The MLN appeared to have the strongest relationship with Zn throughout the season. Defoliation was significantly correlated after the first rating date, and CM (% lvs/tree) was most highly correlated on July 24 and August 16. The % lvs/tree exhibiting NLS was less correlated with Zn deposition from July 3 to August 16, probably due to increased defoliation.

4. DISCUSSION

Black cherry and black locust were highly sensitive to concentrations of HC obscurant used in this study. Conversely, even after four relatively high doses of obscurant, all three conifer species and sweet gum remained asymptomatic. Zinc deposition has been offered as the measure of dose

within this report, but foliar symptoms mimicked Cl toxicity because they progressed from chlorotic mottle to marginal chlorosis and eventual necrosis with increasing dose over the entire exposure sequence [11]. Foliar analyses are in progress to determine if Zn and/or Cl are present at phytotoxic levels.

Exposures using HC obscurant within open-top chambers were sufficient to injure two forest tree species significantly. Foliar injury on these species increased as obscurant concentration increased, but no seedling mortality occurred even at the highest exposure. Trees in this study were exposed to the canisters at their points of ignition, a highly unlikely event in military field training. However, these canisters contained much less reactant (539 g) than that used in field-training smokepots (4,535 g or 13,600 g). In addition, continuous exposure times of the small canisters averaged 3 min compared with an average of 16 min for several smokepots in the field [9]. Therefore, the exposures in the present study are somewhat elevated but not unreasonable.

5. CONCLUSIONS

Significant foliar injury was observed on two of eight tree species exposed to HC obscurant within open-top chambers. Sensitivity was greatest on black locust and black cherry. These two species should be considered bioindicators for this obscurant. Longer term studies and more repeated dosing may produce injury on other test species. Zinc deposition was highly correlated with most symptoms on the sensitive species, although Zn is not necessarily the toxicant. Tissue analyses may indicate another cause of foliar injury.

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